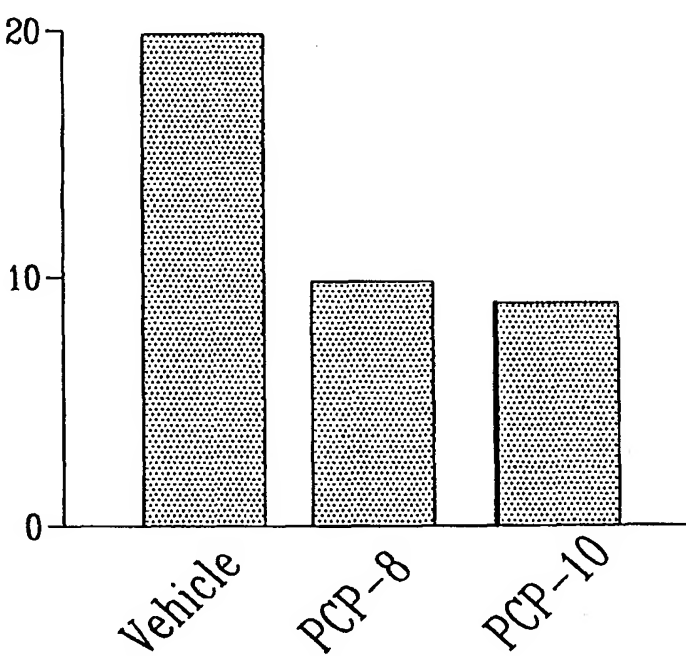




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<b>(21) International Application Number:</b> PCT/CA99/00844 <b>(22) International Filing Date:</b> 15 September 1999 (15.09.99) <b>(30) Priority Data:</b> 09/154,627 17 September 1998 (17.09.98) US <b>(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application</b> US 09/154,627 (CIP) Filed on 17 September 1998 (17.09.98) <b>(71) Applicant (for all designated States except US):</b> HOPITAL SAINTÉ-JUSTINE [CA/CA]; 3175 Côte Sainte-Catherine, Montréal, Québec H3T 1C5 (CA). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> CHEMTOB, Sylvain [CA/CA]; 6885 Banting, Montréal, Québec H4W 1G1 (CA). PERI, Krishna, G. [CA/CA]; #315, 3555 Atwater Avenue, Montréal, Québec H3H 1Y3 (CA). <b>(74) Agents:</b> CÔTÉ, France et al.; Swabey Ogilvy Renault, Suite 1600, 1981 McGill College Avenue, Montréal, Québec H3A 2Y3 (CA).		<b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>								
<b>(54) Title:</b> G PROTEIN-COUPLED RECEPTOR AGONISTS <sup>5</sup> OR ANTAGONISTS										
<b>(57) Abstract</b> <p>The present invention relates to a new class of G protein-coupled receptor agonist or antagonist, which specifically binds to the receptor protein structural elements, thus altering signal transmission and subsequent physiological effects. Described herein are peptide sequences derived from the G protein-coupled receptor protein, produced by chemical methods as selective inhibitors of signal transduction associated with stimulation of the receptor by its ligand. Such peptides or molecules derived from their primary, secondary or tertiary structures may be used as effective tocolytics for the prevention of premature labor or be used for the treatment of dysmenorrhea.</p> <div style="display: flex; align-items: center; justify-content: center;"> <div style="writing-mode: vertical-rl; transform: rotate(180deg); text-align: center; margin-right: 10px;">       Fold stimulation of IP hydrolysis     </div>  <table border="1" style="margin-left: 20px;"> <caption>Data from Bar Chart</caption> <thead> <tr> <th>Condition</th> <th>Fold stimulation of IP hydrolysis</th> </tr> </thead> <tbody> <tr> <td>Vehicle</td> <td>20</td> </tr> <tr> <td>PCP-8</td> <td>10</td> </tr> <tr> <td>PCP-10</td> <td>~9</td> </tr> </tbody> </table> </div> <p style="text-align: center; margin-top: 10px;">100 µM</p>			Condition	Fold stimulation of IP hydrolysis	Vehicle	20	PCP-8	10	PCP-10	~9
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G PROTEIN-COUPLED RECEPTOR AGONISTS OR ANTAGONISTSBACKGROUND OF THE INVENTION(a) Field of the Invention

5           The invention relates to development of agonist or antagonist of a G protein-coupled receptor, which bind to the G protein-coupled receptor from the extra-cellular side in a manner different from that of the natural ligand.

10   (b) Description of Prior Art

Prostaglandins are derived from the oxygenation of arachidonic acid by prostaglandin synthetases. Prostaglandins mediate a wide variety of physiological actions, such as vasomotricity, sleep/wake cycle, 15 intestinal secretion, lipolysis, glomerular filtration, mast cell degranulation, neurotransmission, platelet aggregation, leuteolysis, myometrial contraction and labor, inflammation and arthritis, patent ductus arteriosus, cell growth and differentiation (Coleman, R.A. 20 et al., 1994, *Pharmacol. Rev.* 46:205-229; Goetzl, E.J. et al., 1995, *FASEB J.* 9:1051-10585). Prostanoids mediate their actions through binding to distinct receptors, which belong to the super family of rhodopsin-like seven transmembrane helical receptors. These 25 receptors are coupled to heterotrimeric G-proteins comprising of  $\alpha$ ,  $\beta$  and  $\gamma$  subunits which, upon activation, elicit alterations in cell calcium, initiate phosphoinositide hydrolysis or promotion or repression of cyclic adenosine monophosphate synthesis (Strader C. D. 30 et al., 1994, *Ann. Rev. Biochem.* 63:101-132).

Of the five pharmacologically distinct prostanoid receptors for PGE<sub>2</sub>, PGI<sub>2</sub>, PGD<sub>2</sub>, PGF<sub>2 $\alpha$</sub>  and TxA<sub>2</sub> and their many isoforms, the receptor for PGF<sub>2 $\alpha$</sub> , also called FP receptor, shows limited tissue distribution, pre- 35 dominantly expressed in corpora leutea, uterine myome-

trium, trabecular meshwork of the eye, and to a lesser extent in vascular smooth muscle. Initiation of labor is marked by tremendous rise in  $\text{PGF}_{2\alpha}$  levels and increased uterine contractility. The wide spread use of

5  $\text{PGF}_{2\alpha}$  analogues to induce labor in veterinary industry points to the primary role of  $\text{PGF}_{2\alpha}$  and its receptor in parturition. This is underscored by the fact that mice lacking the FP receptor fail to undergo labor (Sugimoto et al., 1997, *Science* 277:81-83). In face of escalating

10 costs incurred as a result of premature births and associated complications to the neonate, such as intra-ventricular hemorrhage, bronchopulmonary displasia and periventricular leukomalacia leading to cerebral palsy, prolongation of gestation by arresting premature labor

15 is an effective preventive therapy. The relative success of nonsteroidal anti-inflammatory drugs as a short-term therapy toward prevention of premature labor is based on their inhibitory actions upon the synthesis of prostaglandins, particularly  $\text{PGE}_2$  and  $\text{PGF}_{2\alpha}$ . However,

20 inhibition of  $\text{PGE}_2$  is associated with serious complications to the fetus such as the closure of ductus arteriosus, renal failure and pulmonary hypertension.

At another level,  $\text{PGF}_{2\alpha}$  has been attributed a major role in dysmenorrhea, a condition which afflicts

25 5%-7% of premenopausal women. A pre-menstrual increase in  $\text{PGF}_{2\alpha}$  levels resulting in myometrial spasms underlies the pathogenesis of this disorder. Lack of effective antagonists of FP receptor for extended therapy hampered the advances in preventing premature labor and

30 associated sequelae, and the design of such antagonists is the subject of this application.

Human FP receptor is a 45 kDa integral membrane glycoprotein, consisting of 359 amino acids and shares only 47% sequence identity with  $\text{EP}_1$  receptor, and to a

35 lesser extent with other prostanoid receptors

(Abramovitz et al., 1994, *J. Biol. Chem.* 269:2632-2636). Binding of  $\text{PGF}_{2\alpha}$  to FP receptor is followed by the activation of  $\text{G}_{\alpha\beta\gamma}$  complex, increased GTP binding by the  $\text{G}_{\alpha}$  subunit, stimulation of phospholipase  $\text{C}\beta$  activity, release of inositol phosphates, increased intracellular calcium and subsequent signal transduction phenomena ultimately leading to smooth muscle contraction (Coleman, R.A. et al., 1994, *Pharmacol. Rev.* 46:205-229). The FP receptor is the only efficacious target for development of therapeutic drugs since a few  $\text{G}_{\alpha}$ -proteins catalyze the actions of hundreds of G-protein coupled receptors, thus targets downstream from the receptor are essentially of little use.

Antagonists of FP receptors directed to the ligand binding site could be of limited use since ligand based inhibitors show cross reactivity with other prostanoid receptors. Their efficacy will be compromised in face of tremendous increase in  $\text{PGF}_{2\alpha}$  concentrations in myometrium at the onset of labor and in menstruation. The high basal activity of the receptors in the absence of ligand limits the use of ligand-based inhibitors.

It would be highly desirable to be provided with agonist or antagonist of FP receptors, which do not crossreact with other prostanoid receptors, and are effective even in the presence of excess ligand.

#### SUMMARY OF THE INVENTION

One aim of the present invention is to provide agonist or antagonist of FP receptors, which do not crossreact with other prostanoid receptors.

Another aim of the present invention is to provide activators or inhibitors of FP receptors by a novel strategy to target the extracellular domains of the receptor protein.

In accordance with the present invention, there is provided a G protein-coupled receptor agonist or antagonist which specifically binds to the juxtamembrane extracellular structural elements of the G protein-coupled receptor in a manner different from that of the natural ligand, and wherein said agonist or antagonist alter the transduction of an intracellular signal. The G protein-coupled receptor agonist or antagonist may be derived from the amino acid sequence of the receptor.

In accordance with a preferred embodiment of the present invention, the agonist or antagonist does not crossreact with other prostanoid receptors.

The antagonist is effective in the presence of excess ligand.

The agonist or antagonist may preferably comprise an amino acid sequence derived from the first and/or second extracellular loops of prostanoid receptors.

In accordance with another embodiment of the present invention, the antagonists of the present invention comprise amino acid sequences derived from the first and second extracellular loops of prostanoid receptors.

In accordance with a preferred embodiment of the present invention, the G protein-coupled receptor is the prostaglandin  $F_{2\alpha}$  receptor (FP receptor).

In accordance with a preferred embodiment of the present invention, the antagonist of the present invention comprises amino acid sequences derived from the prostaglandin  $F_{2\alpha}$  receptor.

Preferably, the antagonist include, without limitation, amino acid sequence of the FP receptor selected from the group consisting of ILGHRDYK (PCP-8; SEQ ID NO:1); WEDRFYLL (PCP-10; SEQ ID NO:2); YQDRFYLL

(PCP-14; SEQ ID NO:3); ILAHRDYK (PCP-13.7; SEQ ID NO:4); ILGFRDYK (PCP-13.11; SEQ ID NO:5); ILGHKDYK (PCP-13.13; SEQ ID NO:6); ILGHRNYK (PCP-13.14; SEQ ID NO:7); ILGHQDYK (PCP-13.18; SEQ ID NO:8); ILGHRDY-amide  
5 (PCP-13.20; SEQ ID NO:9); ILGHRDYK-amide (PCP-13.21; SEQ ID NO:10); ILGWRDYK (PCP-13.22; SEQ ID NO:11); ILGXRDYK (PCP-13.24; SEQ ID NO:12) protein fusions and peptidomimetics thereof; wherein said amino acid sequence contains L-  
10 and/or D-amino acid.

In accordance with the present invention, there is provided a peptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1 to 12 and wherein said amino acid sequence contains L- and/or  
15 D-amino acid, an amino acid sequence with at least about 90% homology to SEQ ID NO:1 to 12, and peptidomimetic thereof.

In accordance with the present invention, there is provided a pharmaceutical composition containing at  
20 least a G protein-coupled receptor agonist and antagonist of the present invention, mixture thereof, or functional derivatives thereof in association with a pharmaceutically acceptable carrier.

In accordance with another embodiment of the  
25 present invention, there is provided a method for preventing premature delivery of fetus, which comprises the step of administering to a female in need of such a treatment a therapeutically effective amount of a G protein-coupled receptor antagonist or functional  
30 derivatives thereof, wherein the antagonist or functional derivatives thereof specifically binds to the extracellular face of the receptor, thereby hampering uterine contractions.

In accordance with another embodiment of the  
35 present invention, there is provided a method for pre-

venting and/or treating dysmenorrhea comprising the step of administering to a female in need of such a treatment a therapeutically effective amount of a G protein-coupled receptor antagonist or functional derivatives thereof, wherein the antagonist or functional derivatives thereof specifically binds to the extracellular face of the receptor to hamper transduction of a signal thereby reducing the pain associated with contractions.

10 In accordance with another embodiment of the present invention, there is provided a method of identifying a compound as a G protein-coupled receptor agonist or antagonist capable of binding to the extracellular elements of the said receptor in a manner different from that of the natural ligand, comprising the steps of:

- 20 a) culturing cells which express said receptor or identifying animal tissues *ex vivo* or *in vivo* where physiological consequences are dependent on said receptor;
- b) contacting said cells or tissues with said compound to be tested for agonist or antagonist activity at said receptor; and
- 25 c) measuring a response to alter the transduction of a signal resulting in physiological consequences selected from the group consisting of increments in cell calcium, phosphoinositide hydrolysis, increased/decreased cellular cyclic adenosine monophosphate, cell growth and/or differentiation, altered gene expression, and
- 30 smooth muscle contraction or dilation, wherein said response is indicative of agonist or antagonist activity.

35 In accordance with another embodiment of the present invention, there is provided a method of iden-



tifying a compound as a prostaglandin F<sub>2</sub> alpha receptor agonist or antagonist capable of binding to the extracellular elements of the said receptor in a manner different from that of the natural ligand, comprising the steps of:

- a) culturing cells which express said receptor or identifying animal tissues *ex vivo* or *in vivo* where physiological consequences are dependent on said receptor;
- 10 b) contacting said cells or tissues with said compound to be tested for agonist or antagonist activity at said receptor; and
- c) measuring a response to alter the transduction of a signal resulting in physiological consequences selected from the group consisting of  
15 increments in cell calcium, phosphoinositide hydrolysis, cell growth and/or differentiation, altered gene expression, and smooth muscle contraction or dilation, wherein said response is  
20 indicative of agonist or antagonist activity.

For the purpose of the present invention the following terms are defined below.

The expression "a G protein-coupled receptor agonist or antagonist" is intended to mean any natural  
25 or synthetic compound, peptide protein, antibody, peptidomimetic or small chemical molecules, without limitation, insofar as it can specifically bind to the extracellular structural elements of the G protein-coupled receptor to alter transduction of a signal. More  
30 preferably, the agonist or antagonist does not crossreact with other prostanoid receptors.

The expression "functional derivatives" of G protein-coupled receptor agonist or antagonist is intended to mean mimetic compounds and/or structurally  
35 unrelated compounds with respect to G protein-coupled

receptor antagonist, which can also specifically bind to the extracellular structural elements of the G protein-coupled receptor to alter transduction of a signal.

5           The expression "peptidomimetic thereof" is intended to mean any chemical entities, mimetic compounds and/or structurally unrelated compounds with respect to G protein-coupled receptor agonist or antagonist, which can also specifically bind to the  
10       extracellular structural elements of the G protein-coupled receptor to alter transduction of a signal.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

15           Fig. 1 illustrates the inhibitory effects of PCP-8 and PCP-10 on FP receptor function upon stimulation with  $\text{PGF}_{2\alpha}$  in accordance with the embodiment of the present invention;

20           Fig. 2A illustrates the effects of PCP-8 and PCP-10 on the diameter of the microvessels of pig retina upon stimulation with either  $\text{PGF}_{2\alpha}$  or thromboxane  $\text{A}_2$  mimetic, U46619;

            Fig. 2B illustrates the dose response of  $\text{PGF}_{2\alpha}$  on the diameter of pig microvessels treated previously with PCP-8 or PCP-10;

25           Fig. 2C illustrates the effects of thromboxane  $\text{A}_2$  mimetic, U46619, on the diameter of pig microvessels treated previously with PCP-8 and PCP-10;

            Fig. 3A illustrates the effects of PCP-10 upon spontaneous contractions of uterine smooth muscle;

30           Fig. 3B illustrates the dose response of prostaglandin  $\text{F}_{2\alpha}$  in the presence/absence of PCP-8 and PCP-10 upon uterine smooth muscle contraction; and

            Fig. 4 illustrates the reversal of basal tone of bovine myometrium even in the presence of FP receptor  
35       ligand,  $\text{PGF}_{2\alpha}$ .

**DETAILED DESCRIPTION OF THE INVENTION**

In accordance with the present invention, there is provided a new class of G protein-coupled receptor antagonists, which bind to the extracellular molecular surface, thus hamper signal transduction.

Also provided is a novel strategy to target the extracellular loops of the receptor which contribute to the structural or functional integrity of the receptor. Antagonists thus bind to cognate elements in the extracellular surface of the receptor and prevent the receptor function by interfering with its signal initiation or transduction.

There is provided proof of selectivity of the antagonists to FP receptor by showing an absence of their effects on a related prostanoid receptor for thromboxane A<sub>2</sub>, known as TP receptor which is also involved in smooth muscle contraction.

**Preparation of inhibitors****Chemical synthesis of PCP-8 and PCP-10:**

All peptides which are 8 amino acids in length were synthesized using F-moc chemistry and solid phase Merrifield method two peptides, PCP-8 and PCP-10. These peptides were purified by HPLC and their purity tested by mass spectroscopy.

In accordance with the present invention, a novel strategy of using peptides derived from the extracellular domains of prostaglandin F<sub>2α</sub> receptor, FP, to inhibit the signal transduction and the functional consequences of FP receptor. This method could be generalized to all G protein-coupled receptors. Peptides derived from the first and second extracellular loops of FP receptor were found to be effective inhibitors of FP receptor.

The present invention could be readily understood by referring to the following examples, which are given to illustrate the invention rather than to limit its scope.

5

#### EXAMPLE I

##### **Effects of peptides, PCP-8 and PCP-10, on ligand-induced phosphoinositide hydrolysis in mammalian cells overexpressing the FP receptor**

Both PCP-8 and -10 were tested in HEK293 cells expressing the human FP receptor. For this purpose, HEK 293 cells stably expressing human FP receptor were plated in 12-well plates in DMEM medium containing 10% fetal bovine serum, penicillin (10 U/ml) and streptomycin (10  $\mu$ g/ml) and cultured in a humidified atmosphere containing 5% CO<sub>2</sub> at 37°C. After the wells were 80% confluent, the cells were labeled with 2  $\mu$ Ci/ml of [<sup>3</sup>H]-myo inositol overnight. Next day, the cells were washed once with PBS, and incubated in 0.5 ml of Kreb's buffer containing 10 mM LiCl and indicated concentrations of PCP peptides for 30 min. PGF<sub>2 $\alpha$</sub>  at 1  $\mu$ M was added to the cells and the incubation was carried out for an additional 30 min. The cells were solubilized with 0.1 N NaOH for 10 min and neutralized with 0.1 N formic acid. The lysates were collected and 1 ml each of methanol and chloroform were sequentially added and vortexed briefly. After centrifugation to separate the phases, inositol phosphates were separated by ion exchange chromatography as described below (Berridge, M.J. et al., 1983, *Biochem. J.* **212**:473-482).

Briefly, the medium was discarded and the IP<sub>3</sub> synthesis was stopped by adding 0.6 ml ice-cold methanol. The cells were scraped and collected into polypropylene tubes. Distilled water (0.5 ml) and chloroform (0.6 ml) were added and vigorously vortexed for 2 min. The phases were separated by centrifugation at 6000 x g

35

for 10 min. The aqueous phase was applied to AG-1X-8™ (Formate form) anion exchange columns (1 ml bed volume) and free inositol was eluted with 10 ml of water, followed by 60 mM ammonium formate in 0.1 M formic acid. Then, the inositol phosphates were eluted with 5 ml of 1.2 M ammonium formate in 0.1 M formic acid. After adding 3 volumes of scintillation cocktail (Optiphase-HiSafe III), the eluates were counted by scintillation spectrophotometry.

The results of these experiments are shown in Fig. 1. Data are expressed as fold stimulation of inositol phosphate synthesis by 1  $\mu$ M PGF<sub>2 $\alpha$</sub>  compared to the unstimulated controls. Both PCP-8 and -10 at 100  $\mu$ M potently inhibited inositol phosphate synthesis initiated by the action of PGF<sub>2 $\alpha$</sub>  on FP receptor. The half maximal inhibitory concentrations for both PCP-8 and -10 were slightly less than 100  $\mu$ M.

#### EXAMPLE II

##### **Testing PCP peptides in porcine eyecup model of ex vivo vasomotricity assay**

In order to see if the peptides could inhibit FP function using an ex vivo model, we chose porcine eyecup model, an ex vivo assay of vascular constriction in porcine retinas which we previously described and validated (Li et al., 1996 J. Pharmacol. Expt. Therapeut. 278: 370-377; Li et al., 1997 Am. J. Physiol. 273: R1283-90; Abran et al., 1997 Am. J. Physiol. 272: R995-1001). Since FP receptor densities in newborn vasculature are minimal due to down regulation by high levels of circulating prostaglandins in the perinatal period, the newborn pigs were treated with a prostaglandin synthetase blocker, ibuprofen (30 mg/Kg of bodyweight/ 8 h for 24 h) to increase the density of the receptors as well as their vasomotor effects. By inhibiting circulating prostaglandins, we were able to show potent

inhibition of FP receptor-mediated second messenger synthesis as well as FP-mediated vascular constriction in this eyecup model.

To prepare eyecups, a circular incision was made  
5 3-4 mm posterior to ora serrata to remove the interior segment and vitreous body with minimal handling of the retina. The remaining eyecup was fixed with pins to a wax base in a 20 ml tissue bath containing 20 ml of  
10 Krebs's buffer (pH 7.35-7.45), protease inhibitors, leupetin and aprotinin (10  $\mu$ g/ml each), and equilibrated with 21% oxygen and 5% carbon dioxide at 37°C. The preparations were allowed to stabilize for 30 min. Peptides at 100  $\mu$ M were added and incubation was continued for 30 min before the addition of PGF<sub>2 $\alpha$</sub> .

15 Cumulative concentration-responses of PGF<sub>2 $\alpha$</sub>  and TxA<sub>2</sub> mimetic, U46619, ( $10^{-10}$  to  $10^{-5}$  M) curves were constructed separately. To assess the reversibility of the antagonists, the eyecups were thoroughly washed (which would wash away the peptide) with incubation medium and  
20 concentration response curves for PGF<sub>2 $\alpha$</sub>  were determined. The outer vessel diameter was recorded with a video camera mounted on a dissecting microscope (Zeiss M 400™) and the responses were quantified by a digital image analyzer (Sigma Scan Software, Jandel Scientific,  
25 Corte Madera, CA). Vascular diameter was recorded before and 10 min following the topical application of the agent. Each measurement was repeated three times and showed <1% variability.

The results are shown in Fig. 2. The peptide  
30 PCP-10 had no effect on the basal tone (diameter of the microvessel) of the vessel (Fig. 2A; left panels). Addition of 1  $\mu$ M of PGF<sub>2 $\alpha$</sub>  potently constricted the vessel in the absence of the peptide (middle-top panel), whereas presence of PCP-10 at 100  $\mu$ M markedly inhibited  
35 PGF<sub>2 $\alpha$</sub> -mediated vasoconstriction (middle-bottom panel).

The peptide had no effect on the vasoconstriction effected by 1  $\mu$ M TxA<sub>2</sub> mimetic, U46619, (right panels) acting on another prostanoid receptor coupled to constriction, namely TP receptor. Similar results were  
5 obtained for PCP-8 as well. A dose response of PGF<sub>2 $\alpha$</sub>  on the vascular diameter in the presence/absence of PCP-8 and PCP-10 peptides are presented in Fig. 2B. Both peptides abrogated the vasomotor responses even at concentrations exceeding 1 $\mu$ M of PGF<sub>2 $\alpha$</sub> , suggesting, as  
10 expected, that the peptides may be acting in a non-competitive fashion. However, the peptides had no effect on vasoconstriction produced by thromboxane A<sub>2</sub> (Fig. 2C).

Similarly, a peptide derived from the first  
15 extracellular loop of FP receptor, PCP-15, inhibited PGF<sub>2 $\alpha$</sub> -induced constriction (10<sup>-7</sup>M) (88.1% over untreated control; Table 1).

### EXAMPLE III

#### 20      **Testing peptide variants of PCP-8 in porcine eyecup model of ex vivo vasomotricity assay**

In order to understand the structural requirements of PCP-8 in its inhibitory action on PGF<sub>2 $\alpha$</sub> -induced vasoconstriction, different amino acids in PCP-8 sequence  
25 were replaced with other D- or L- amino acids and the resulting peptides were chemically synthesized and tested in porcine eyecup model of ex vivo vasomotricity assay. These peptide variants are listed in Table 1.

Table 1

Amino acid sequences of peptide variants of PCP-8 and their inhibitory potency in porcine eyecup model of *ex vivo* vasomotricity assay

Peptide PCP-	%Vasomotor response (of max. constriction) <sup>1</sup>	% inhibition of maximal response <sup>2</sup>	Peptide sequence	SEQ ID NO:
8	50.0	50.0	ilghrdyk	1
10	20.0	80.0	wedrfyll	2
14	36.0	64.0	YQDRFYLL	3
13	20.0	80.0	ILGHRDYK	1
13.7	23.8	76.2	ILAHRDYK	4
13.8	46.8	53.2	ILaHRDYK	4
13.11	13.0	87.0	<u>ILGFRDYK</u>	5
13.13	36.9	63.1	ILGHKDYK	6
13.14	40.3	59.7	ILGHRNYK	7
13.18	30.0	70.0	ILGHQDYK	8
13.20	49.6	50.4	ILGHRDY-amide	9
13.21	46.2	53.8	ILGHRDYK-amide	1
13.22	16.6	83.4	ILGWRDYK	10
13.24	6.2	93.8	ILGXRDYK	11
15	11.9	88.1	SNVLC SIF	12

5           <sup>1</sup>Percent vasomotor response in the presence of 100  $\mu$ M peptide is calculated as percent change in average vascular diameter produced by  $10^{-7}$  M  $\text{PGF}_{2\alpha}$  to the eyecup in the presence of the peptide compared to maximal constriction observed in the absence of the peptide.

10           <sup>2</sup>Percent inhibition produced by each peptide is calculated as (100-per cent vasomotor response).

Small letters indicate L-amino acids and capital letters indicate D- amino acids. I = isoleucine; L= leucine; G =glycine; H=histidine; R=Arginine; D=Aspartic acid; Y=Tyrosine; K=Lysine; A=Alanine; W= Tryptophan; E=Glutamic acid; F= Phenyl alanine; Q=Glutamine; N=Asparagine; P=Proline; S=Serine; X=Cyclohexyl alanine. Peptides were dissolved in DMSO  
 15 freshly just before the experiment as 10 mM stocks and added to the eye cups 30 min before the addition of  
 20  $10^{-7}$  M  $\text{PGF}_{2\alpha}$ .



A total of 25 variants of PCP-8 were synthesized and the efficacious or potent peptides are listed in Table 1. These peptides incorporate L- to D-amino acid changes, deletions, subtle variations in aromaticity, hydrogen bond donor status as opposed to ionic interactions and hydrophobicity. These peptides were tested at 100  $\mu$ M concentration in porcine retinal vasomotricity assay and the results are summarized in Table 1.

The results are summarized as follows:

1. Converting all L-amino acids of PCP-8 to D-amino acids (PCP-13) increased the inhibitory potency dramatically. Removal of N-terminal hydrophobic dipeptide sequence from either PCP-8 (PCP-11) or PCP-10 (PCP-12) resulted in significant reduction in the inhibitory action of the peptides.
2. Glycine to alanine (13.7) does not change the activity of PCP-13, whereas change to proline (13.16), L-alanine (13.8), or deletion of the residue (13.17) entirely resulted in loss of activity. Glycine is an important linker residue separating the HRD motif from the IL hydrophobic sequence.
3. HRD-motif is important for the activity of PCP-13. Alanine substitutions (13.1-13.3) or to change to L-configuration (13.4-13.6) resulted loss of inhibitory activity of PCP-13. Aromaticity of His is more important than the positive charge, since H to F (13.11) or W (13.22) or X (13.24), but not to Y (13.23), did not result in significant reduction of peptide inhibitory potency. Side chain length appears to be more critical in case of D residue than R; changing D to E (13.12) resulted in loss of half of the inhibitory activity whereas R to K (13.13) or to Q (13.18) affected the activity of PCP-13 moderately. D to N (13.14) resulted in moderate loss of activity,

whereas a serine substitution (13.19) lead to drastic loss of activity of PCP-13.

4. Deletion of terminal lysine (13.15) or substitution with W (13.9) resulted in complete loss of activity; however, conversion of terminal carboxylate into an amide (13.20 & 13.21) resulted in moderate gain of activity of the peptide inhibitor. Substitution of aromatic residue, Y, with E (13.10) completely abolished the inhibitory potency of PCP-13.

Thus the structure of PCP-13 in D-configuration appears to consists of a N-terminal hydrophobic anchor spaced from the central HRD motif by a glycine residue possibly resulting in a turn conformation of the active peptide; Aromatic and hydrophobic interactions at the carboxy terminus may also add to the potency of PCP-13.

#### EXAMPLE IV

##### **Testing PCP peptides in porcine uterine strip of ex vivo basal contraction assay**

In ex vivo experiments using porcine uterine strips, the peptides were able to prevent both basal and PGF<sub>2α</sub>-induced contraction.

Uterine tissues from non-pregnant adult pigs were obtained from a local slaughter house and transported to the laboratory on ice. Uterine myometrial strips of approximately 1 cm in length were set up in organ baths containing Kreb's buffer equilibrated with 21% oxygen at 37°C as we have described (Potvin, W. et al., 1990, *Br. J. Pharmacol.* **100**:341-347; Varma, D.R. and Chemtob, S., 1993, *J. Pharmacol. Expt. Ther.* **265**:1096-1104). Contractions were recorded by force transducers on Grass-polygraph. Strips were incubated with or without 100 μM peptides for 30 min before adding PGF<sub>2α</sub> in step-wise increments (10<sup>-9</sup> to 10<sup>-6</sup> M). Data were expressed as percentage increase over the basal level of average tension (g).

A graph of spontaneous uterine contractions (known to be dependent upon prostanoids, mainly  $\text{PGF}_{2\alpha}$ ) in the absence and the presence of  $100\ \mu\text{M}$  PCP-8 are shown in Fig. 3A. Addition of peptide to the strips  
5 reduced the force of basal contraction. A dose response of  $\text{PGF}_{2\alpha}$  on uterine contractility in the presence or absence of PCP-8 and PCP-10 peptides is shown in Fig. 3B. More than 60% (PCP-8) and 80% (PCP-10) reduction in uterine contraction was observed in all concentrations of  $\text{PGF}_{2\alpha}$  tested. Thus, both these peptides  
10 reduced spontaneous as well as  $\text{PGF}_{2\alpha}$ -induced contractions in the uterine strips.

#### EXAMPLE V

##### 15    **Testing PCP peptides in bovine uterine strip of ex vivo basal contraction assay**

Uterine tissues from non-pregnant adult bovine animals were obtained from a local slaughter house and transported to the laboratory on ice. Uterine myometrial strips of approximately 1 cm in length were set  
20 up in organ baths containing Krebs' buffer equilibrated with 21% oxygen at  $37^\circ\text{C}$  as described above. Contractions were recorded on Grass-polygraph by force transducers. Strips were incubated with or without  $100\ \mu\text{M}$  peptides before adding  $\text{PGF}_{2\alpha}$  in step-wise increments ( $10^{-8}$  to  $10^{-6}$  M). Data were expressed as change in basal level of average tension (g). The results are shown in Fig. 4. Application of PCP-10 peptide at  $100\ \mu\text{M}$  reversed the basal tone (contractile state) of the  
25 uterine muscle. Addition of  $\text{PGF}_{2\alpha}$  up to  $10\ \mu\text{M}$  did not affect the relaxation produced by PCP-10 suggesting that the effects of PCP peptides are independent of the ligand, which was also shown in the previous results.

While the invention has been described in connection with specific embodiment thereof, it will be  
35 understood that it is capable of further modifications

and this application is intended to cover any variations, uses or adaptations of the invention following in general, the principles of the invention and including such departures from the present disclosure as come  
5 within the known customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A G protein-coupled receptor agonist or antagonist which specifically binds to the juxtamembrane extracellular structural elements of the G protein-coupled receptor in a manner different from that of the natural ligand, and wherein said agonist or antagonist alters the transduction of an intracellular signal.
2. The agonist or antagonist of claim 1, wherein said agonist or antagonist does not crossreact with other prostanoid receptors and wherein said antagonist is effective in the presence of excess ligand.
3. The agonist or antagonist of claim 1, which comprises an amino acid sequence derived from derived from the first and/or second extracellular loops of prostanoid receptors.
4. The agonist or antagonist of claim 3, which comprises an amino acid sequence derived from the first and second extracellular loop of prostanoid receptors.
5. The antagonist of claim 1, wherein the receptor is prostaglandin F<sub>2α</sub> receptor (FP receptor).
6. The antagonist of claim 2, which comprises amino acid sequence of the FP receptor selected from the group consisting of ILGHRDYK (PCP-8; SEQ ID NO:1); WEDRFYLL (PCP-10; SEQ ID NO:2); YQDRFYLL (PCP-14; SEQ ID NO:3); ILAHRDYK (PCP-13.7; SEQ ID NO:4); ILGFRDYK (PCP-13.11; SEQ ID NO:5); ILGHKDYK (PCP-13.13; SEQ ID NO:6); ILGHRNYK (PCP-13.14; SEQ ID NO:7); ILGHQDYK (PCP-13.18; SEQ ID NO:8); ILGHRDY-amide (PCP-13.20; SEQ ID NO:9); ILGHRDYK-amide (PCP-13.21; SEQ ID NO:1);

ILGWRDYK (PCP-13.22; SEQ ID NO:10); ILGXRDYK (PCP-13.24; SEQ ID NO:11); SNVLCSIF (PCP-15; SEQ ID NO:12); protein fusions and peptidomimetics thereof; wherein said amino acid sequence contains L- and/or D-amino acid.

7. A peptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1 to 12 and wherein said amino acid sequence contains L- and/or D-amino acid, an amino acid sequence with at least about 90% homology to SEQ ID NO:1 to 12, and peptidomimetic thereof.

8. A method for preventing premature delivery of fetus, which comprises the step of administering to a female in need of such a treatment a therapeutically effective amount of a G protein-coupled receptor antagonist of any one of claims 1 to 6 or functional derivatives thereof.

9. A method for preventing and/or treating dysmenorrhea comprising the step of administering to a female in need of such a treatment a therapeutically effective amount of a G protein-coupled receptor antagonist of any one of claims 1 to 6 or functional derivatives thereof.

10. A pharmaceutical composition containing at least a G protein-coupled receptor agonist of any one of claims 1 to 4, an antagonist of any one of claims 1 to 6, mixture thereof, or functional derivatives thereof in association with a pharmaceutically acceptable carrier.

11. A method of identifying a compound as a G protein-coupled receptor agonist or antagonist capable of binding to the extracellular elements of the said receptor in a manner different from that of the natural ligand, comprising the steps of:

- a) culturing cells which express said receptor or identifying animal tissues *ex vivo* or *in vivo* where physiological consequences are dependent on said receptor;
- b) contacting said cells or tissues with said compound to be tested for agonist or antagonist activity at said receptor; and
- d) measuring a response to alter the transduction of a signal resulting in physiological consequences selected from the group consisting of increments in cell calcium, phosphoinositide hydrolysis, increased/decreased cellular cyclic adenosine monophosphate, cell growth and/or differentiation, altered gene expression, and smooth muscle contraction or dilation, wherein said response is indicative of agonist or antagonist activity.

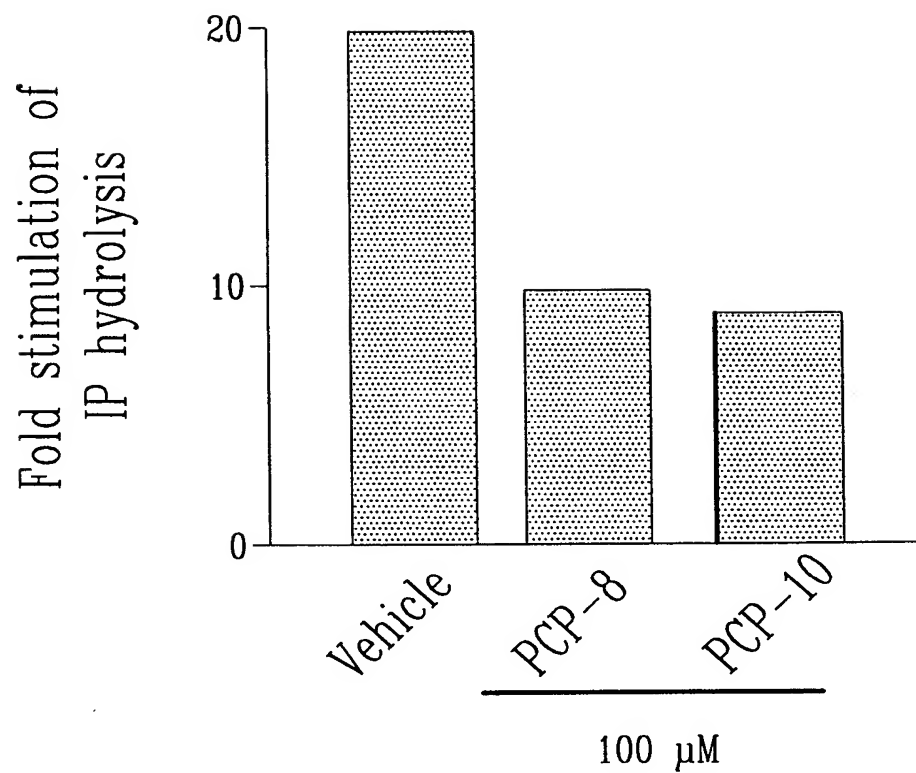
12. A method of identifying a compound as a prostaglandin F<sub>2</sub> alpha receptor agonist or antagonist capable of binding to the extracellular elements of the said receptor in a manner different from that of the natural ligand, comprising the steps of:

- a) culturing cells which express said receptor or identifying animal tissues *ex vivo* or *in vivo* where physiological consequences are dependent on said receptor;
- b) contacting said cells or tissues with said compound to be tested for agonist or antagonist activity at said receptor; and

- c) measuring a response to alter the transduction of a signal resulting in physiological consequences selected from the group consisting of increments in cell calcium, phosphoinositide hydrolysis, cell growth and/or differentiation, altered gene expression, and smooth muscle contraction or dilation, wherein said response is indicative of agonist or antagonist activity.



1/5

FIG. 1

2/5

U46619



PGF<sub>2α</sub>



Basal

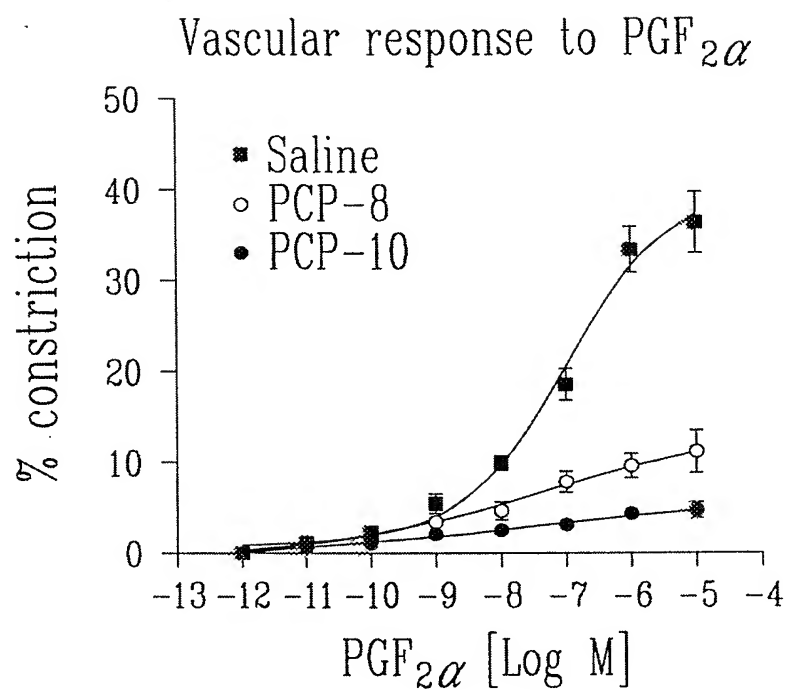
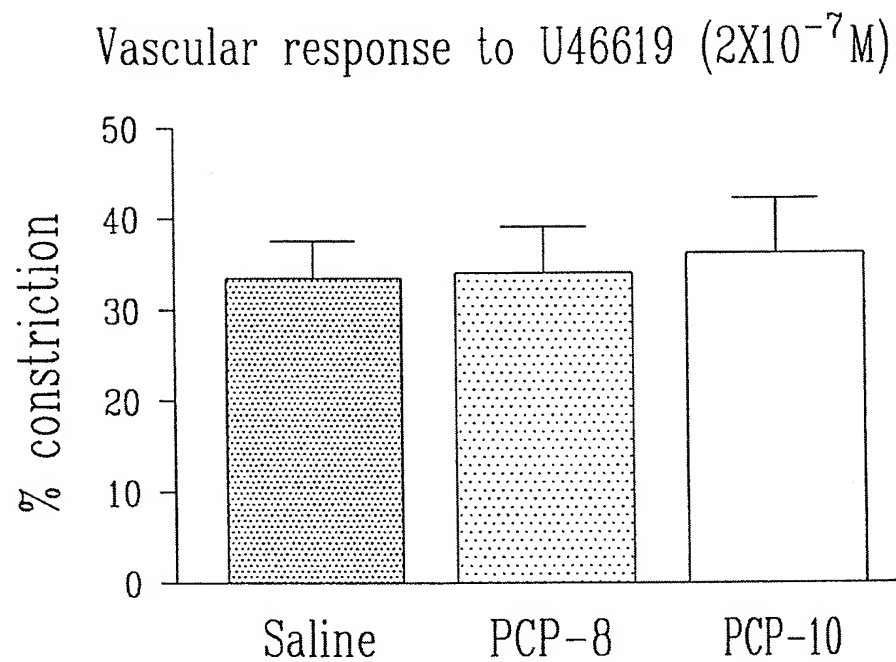


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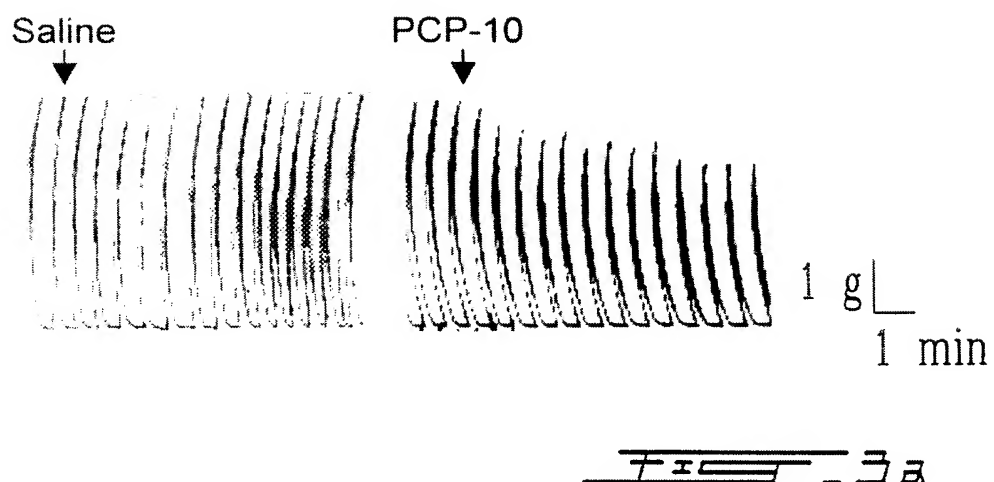
PCP-10

FIG - 2a

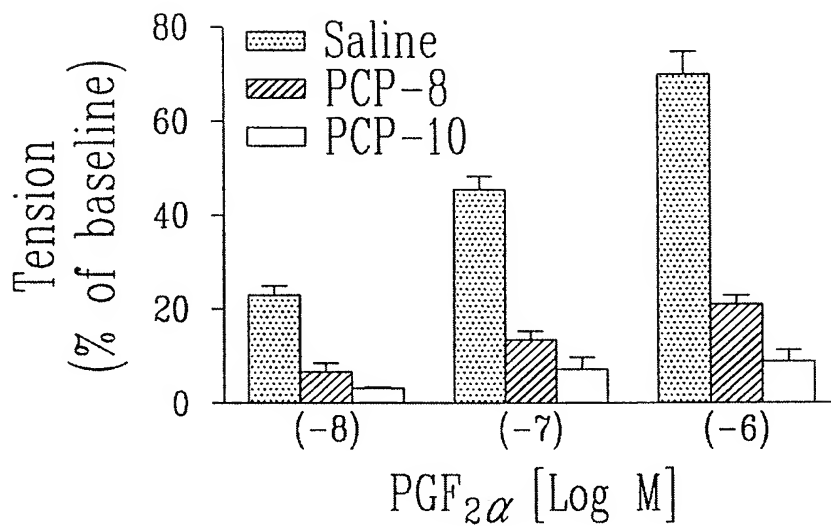
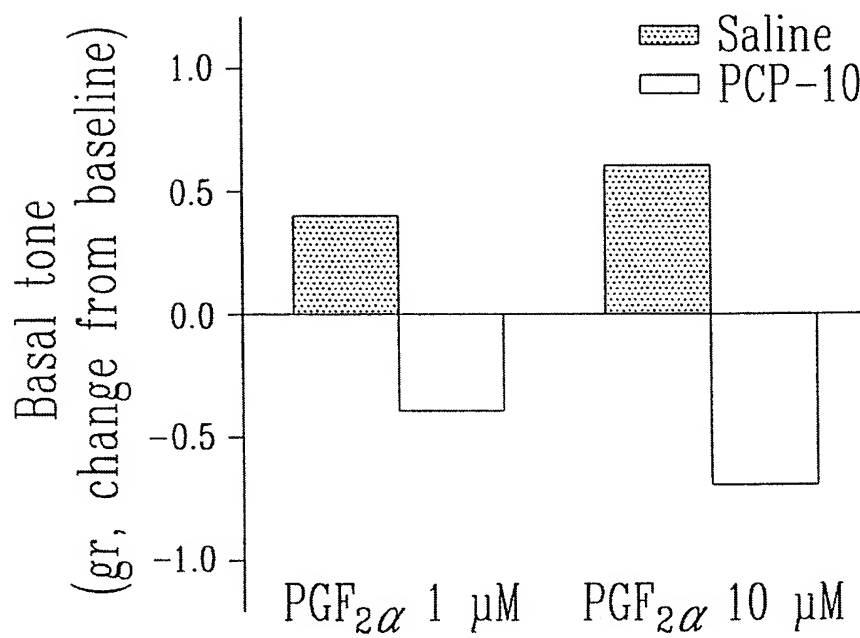
3/5

FIG. 2bFIG. 2c

4/5



5/5

Figure 3bFigure 4

1/3

## SEQUENCE LISTING

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PERI, Krishna G.

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# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/CA 99/00844

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC 7 C12N15/12 C07K14/705 C07K14/72 A61K38/04 A61P15/06 G01N33/50 G01N33/68		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) IPC 7 C07K C12N A61K A61P G01N		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 00551 A (MERCK FROSST CANADA INC ;ABRAMOVITZ MARK (CA); GRYGORCZYK RICHARD) 5 January 1995 (1995-01-05) page 14, line 17 - line 29; claim 8; figures 3,5,6	1,7, 10-12
A	--- WO 96 23225 A (COR THERAPEUTICS INC) 1 August 1996 (1996-08-01) page 17, paragraph 5 page 23 claims 7-21; examples 6,7,11	2-6,8,9
X	--- WO 96 23225 A (COR THERAPEUTICS INC) 1 August 1996 (1996-08-01) page 17, paragraph 5 page 23 claims 7-21; examples 6,7,11	1-4,10, 11
X	--- WO 93 09104 A (SEARLE & CO) 13 May 1993 (1993-05-13) page 8, line 6 - line 19 page 19, line 1 - line 17 page 61 -page 62 claims 36,37	1,2,9-11
--- -/--		
<div style="display: flex; justify-content: space-between;"> <span><input checked="" type="checkbox"/> Further documents are listed in the continuation of box C.</span> <span><input checked="" type="checkbox"/> Patent family members are listed in annex.</span> </div>		
* Special categories of cited documents : <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&amp;" document member of the same patent family</p> </div> </div>		
Date of the actual completion of the international search  <div style="text-align: center; font-weight: bold;">10 February 2000</div>		Date of mailing of the international search report  <div style="text-align: center; font-weight: bold;">01/03/2000</div>
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer  <div style="text-align: center; font-weight: bold;">van Klompenburg, W</div>

# INTERNATIONAL SEARCH REPORT

Intern. Application No  
PCT/CA 99/00844

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 688 938 A (BROWN EDWARD M ET AL) 18 November 1997 (1997-11-18) column 4, line 23 - line 36 column 22, line 48 -column 44, line 28 examples 1-14; tables 2-8 -----	1,2,10, 11
X	KITANAKA ET AL.: "Phloretin as an antagonist of prostaglandin F2alpha receptor in cultured rat astrocytes" JOURNAL OF NEUROCHEMISTRY, vol. 60, no. 2, February 1993 (1993-02), pages 704-708, XP000876615 page 704 page 707 -page 708; figures 1-5; table 1 -----	1,5, 10-12
A	SUGIMOTO ET AL.: "Failure of parturition in mice lacking the prostaglandin f receptor" SCIENCE, vol. 277, 1 August 1997 (1997-08-01), pages 681-683, XP002130206 cited in the application page 681 -page 683; figure 2 -----	8
P,X	GRIFFIN B W ET AL: "AL-8810: a novel prostaglandin F2 alpha analog with selective antagonist effects at the prostaglandin F2 alpha ( FP ) receptor." JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS, (1999 SEP) 290 (3) 1278-84. , XP000876534 page 1282, column 2 -page 1283, column 1; figures 2-8 -----	1,2,5, 8-12

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA 99/ 00844

### Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 8 and 9  
are directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

#### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern. Appl. No.  
PCT/CA 99/00844

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9500551 A	05-01-1995	US 5869281 A	09-02-1999
		AT 170532 T	15-09-1998
		AU 6991894 A	17-01-1995
		CA 2165954 A	05-01-1995
		DE 69413039 D	08-10-1998
		DE 69413039 T	06-05-1999
		EP 0705276 A	10-04-1996
		ES 2120052 T	16-10-1998
		JP 11505701 T	25-05-1999
		US 5840847 A	24-11-1998
WO 9623225 A	01-08-1996	AU 2397399 A	17-06-1999
		AU 701822 B	04-02-1999
		AU 4859996 A	14-08-1996
		CA 2211176 A	01-08-1996
		EP 0870198 A	14-10-1998
		JP 11514207 T	07-12-1999
		NO 973422 A	24-09-1997
		NZ 302524 A	28-05-1999
		US 5763575 A	09-06-1998
		US 5716789 A	10-02-1998
		US 5874400 A	23-02-1999
WO 9309104 A	13-05-1993	AU 2669992 A	07-06-1993
		CA 2118688 A	13-05-1993
		EP 0613472 A	07-09-1994
		JP 7501054 T	02-02-1995
		US 5449674 A	12-09-1995
US 5688938 A	18-11-1997	US 6011068 A	04-01-2000
		AU 709303 B	26-08-1999
		AU 4195796 A	15-05-1996
		BR 9509411 A	30-12-1997
		CA 2202879 A	02-05-1996
		CN 1220658 A	23-06-1999
		CZ 9701182 A	17-09-1997
		EP 0787122 A	06-08-1997
		HU 77980 A	01-02-1999
		JP 11130737 A	18-05-1999
		JP 2882882 B	12-04-1999
		JP 10513436 T	22-12-1998
		NZ 297157 A	30-08-1999
		PL 319812 A	01-09-1997
		WO 9612697 A	02-05-1996
		US 6001884 A	14-12-1999
		US 5858684 A	12-01-1999
		US 5763569 A	09-06-1998
		US 5962314 A	05-10-1999
		WO 9511221 A	27-04-1900
		AU 3122699 A	22-07-1999
		AU 702629 B	25-02-1999
		AU 8087294 A	08-05-1995
		CA 2173747 A	27-04-1995
		CN 1139917 A	08-01-1997
		EP 0724561 A	07-08-1996
		JP 9504032 T	22-04-1997
		SG 52796 A	28-09-1998
		WO 9304373 A	04-03-1993

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 99/00844

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5688938 A		AU 673500 B	14-11-1996
		AU 711247 B	07-10-1999
		AU 7197796 A	20-02-1997
		CA 2115828 A	04-03-1993
		CN 1071333 A	28-04-1993
		EP 0657029 A	14-06-1995
		JP 2860285 B	24-02-1999
		JP 9328420 A	22-12-1997
		JP 2887201 B	26-04-1999
		JP 9281109 A	31-10-1997
		JP 11221095 A	17-08-1999
		JP 2728564 B	18-03-1998
		JP 6510531 T	24-11-1994
		MX 9204881 A	01-04-1993
		NO 940581 A	25-04-1994
		ZA 9206360 A	30-03-1993